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THE BACTERICIDAL AND FUNGICIDAL ACTION OF COPPER SALTS*

STUDIES ON THE BIOCHEMISTRY AND CHEMOTHERAPY OF TUBERCULOSIS, XV

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From the middle of the nineteenth century to the present day the value of copper salts as germicidal or as therapeutic agents has been a subject of interest.

HISTORICAL REVIEW

In 1853 the French government recorded its belief in the harmfulness of copper as a dietary constituent¹ by the passage of a law (afterward repealed) prohibiting its addition to canned vegetables. Apparently the copper served for the maintenance of the green color of the vegetables, tho Johnson and Copeland² suggest that it may have served a double purpose: "Copper sulphate is a powerful germicide. It has for years been added to canned meats and vegetables to prevent fermentation by bacteria and other organisms."

For at least the last quarter of the nineteenth century, the belief in the germicidal action of copper was particularly flourishing. The early observations of its effect were made almost simultaneously on algae, fungi, and bacteria. During the years 1880 to 1883 Burq³ reported the results of an investigation, begun 30 years earlier, comprising a mass of data acquired by personal visits to all sorts of establishments engaged in the manufacture of copper and other metals, by extensive communication with the officers of workmen's unions, working men themselves, proprietors of foundries, physicians, and mayors of towns where metal-working was the principal industry, by correspondence with English, Swedish, and Russian ambassadors, with the directors of Siberian mines, and with all possible individuals engaged in, or connected with, the mining and manufacture of copper—all of which led him to conclude that impregnation of the workmen with copper conferred on them a singular immunity to cholera. He found them equally immune to typhoid infection, and suggested that a similar immunity might exist toward diphtheria and smallpox. (These findings have been largely discredited as a result of more recent investigations.) In 1883 Walker⁴ published a monograph, written 20 years earlier, in which the verdict runs: "At all events the immunity of this class

* Received for publication October 28, 1915.

¹ Kraemer: *Am. Jour. Pharm.*, 1905, 77, p. 265.

² *Jour. Infect. Dis.*, 1905, Suppl. 1, p. 327.

³ *Bull. de l'Acad. de méd.*, 1880, 9, p. 239. *Compt. rend. Acad. d. sc.*, 1882, 95, p. 862. *Compt. rend. Soc. de biol.*, 1883, 5, p. 532.

⁴ *The Prophylactic Power of Copper in Epidemic Cholera*, 1883. Quoted by Moore, *Bull. U. S. Dept. of Agriculture, Bur. Plant Ind.*, 76, p. 52.

of men [copper-workers] for cholera is a remarkable and positive fact. I have for a long time made inquiries in this matter, and cannot as yet learn that a single case has occurred among them." In 1876, according to Green,⁵ von Bucholtz established the fact of the inhibitory effect of copper sulfate, finding that 0.75% checked all bacterial development in nutrient media. Green⁵ himself investigated the disinfectant action of certain soluble copper compounds—the chlorid, nitrate, acetate, aluminate, phenolsulfonate, and sulfate (C. P., crude, and ammoniacal)—on various substances and organisms, and found them efficient, for the most part, in the order given. One hour's exposure to 5% copper chlorid killed cholera and typhoid organisms, while 3 hours' exposure was necessary in the case of *Staphylococcus aureus*. Spore-free anthrax bacilli were killed in 5 minutes by 5% copper chlorid, and anthrax spores were retarded in their development by copper salts, and killed by exposure to 5% copper chlorid for 27 days.

As a fungicide, copper owes its chief prominence to Millardet's use of copper sulfate and lime in the Bordeaux mixture as protection against *Plasmopora*, which wrought such havoc in the French vineyards in 1883. At about the same time Naegeli⁶ observed what he termed the "oligodynamic" effect of copper, one part of which in one billion parts of water was extremely toxic to *Spirogyra*.

From this time on, experimental investigation of the efficiency of copper in various forms seems to have thriven. Behring⁷ found copper sulfate a "very good disinfecting agent." Israel and Klingmann⁸ continued the study of the effect of copper on *Spirogyra*, and on bacteria, using *B. typhosus*, *B. coli*, and *Vibrio cholerae*. In their experiments the bactericidal effect was obtained by exposing the organisms for different lengths of time to water in which small pieces of copper foil had been left for 24 hours. All manifested delay of growth after a half hour's exposure, while 2 hours' exposure killed *B. typhosus*, and 3 hours' exposure, *B. coli* and *V. cholerae*. Increase of temperature doubled the toxic effect, halving the time required. Bolton⁹ found that when metals, and copper among them, were laid on agar plates inoculated with organisms, zones of inhibition were produced. He also discovered that in sufficient dilution copper exerted a stimulative effect. Krönig and Paul¹⁰ in their work on disinfection found that copper chlorid, even in so concentrated a solution as 13%, failed to kill anthrax spores during a 10 days' exposure, tho 3.4% auric chlorid killed them in 33 hours, and 1.69% mercuric chlorid in 14 minutes.

In 1904 the practical use of copper as a disinfectant received a fresh impetus from the publication of investigations which had been prosecuted for 2 years by Moore and his co-workers in the U. S. Department of Agriculture.¹¹ These investigations were directed primarily toward the destruction of those algal growths which produce the various disagreeable tastes and odors so common in many water supplies during the summer months. Moore found small amounts of copper sulfate very efficient in ridding water supplies of algae, while pathogenic bacteria (notably typhoid) also disappeared in many instances. Moore's work aroused widespread experimentation, with varying results. Pen-

⁵ Ztschr. f. Hyg. u. Infektionskrankh. 1895, 13, p. 495.

⁶ Neue Denkschriften der Schweiz. Naturforsch. Gesellschaft., 1893-98, 33-34, 2nd paper.

⁷ Ztschr. f. Hyg. u. Infektionskrankh., 1890, 9, p. 395.

⁸ Virchow's Arch. f. path. Anat., 1897, 147, p. 293.

⁹ Internat. Med. Mag., 1894-5, 3, p. 812.

¹⁰ Ztschr. f. Hyg. u. Infektionskrankh., 1897, 25, p. 1.

¹¹ Bull. U. S. Dept. of Agriculture, Bur. Plant Ind., 64, p. 76.

nington¹² and her assistants found that in very clean brightly polished copper vessels typhoid bacilli were soon killed, sometimes in less than 2 hours, while Kraemer¹, after 2 years of experimentation declared: "I have found that when copper foil is allowed to remain in distilled water from one to five minutes sufficient copper is dissolved by the water to kill typhoid organisms within two hours."

Clark and Gage,¹³ on the other hand, decided that copper sulfate was of little practical importance, since sterilization required amounts so large as to render the water unpalatable. Johnson and Copeland² found reduction of organisms in sewage, both as it was when collected and as it was with the addition of typhoid bacilli. Feldt¹⁴ found that in asparagin media both copper sulfate and copper chlorid inhibited the growth of tubercle bacilli only in concentrations of from 1:5000 to 1:50,000. Von Linden,¹⁵ however, stated that when added to artificial protein-containing media, copper salts exercised an inhibitory effect on vigorous cultures of tubercle bacilli even in dilution of 1:1,000,000. At 1:100,000, all, even large clumps, were killed, so that transfers failed to grow. DeWitt and Sherman,¹⁶ on the other hand, found vigorous growth developing on tubes containing 1 part of copper to 1,000,000 parts of media, while inhibition occurred at a concentration of 1:100,000 and killing only after 24 hours' exposure to 5% copper.

In addition to its use for the purification of drinking water, some attempt has been made at sewage-treatment with copper sulfate, but the excessive amount of organic matter in sewage causes precipitation of the copper, thereby diminishing its efficiency. It has also been employed for the removal of bacteria in swimming pools, more or less successfully.

Since its introduction by Millardet, copper in some form continues a common ingredient of sprays and fungicides. It is frequently employed in sterilization of seeds against adhering or infecting fungi. In this connection it is of interest that one strain of *Penicillium*, a fungus often found contaminating cultures of germinating seeds, is particularly resistant to the action of copper. Pulst¹⁷ found that *P. glaucum* withstood 10% copper sulfate, while Hollrung¹⁸ stated that Trabut had described a *P. cupricum*, (apparently a mutant of *P. glaucum*) which grew in concentrated copper sulfate solution. It may be mentioned in passing that the stock solution of copper amino-acids in this laboratory, containing 1.8% copper, was regularly overgrown with *Penicillium*.

The idea of employing copper as a therapeutic agent has long existed. Burq³ and Walker⁴ recommended the use of copper in the therapy of typhoid. Green⁵ advised the treatment of wounds with copper chlorid. Wilcke¹⁹ used copper acetate internally to free typhoid-carriers of the bacilli, and with some success. Springer and Springer²⁰ found that copper salts delayed or inhibited the action of putrefactive organisms, and so might have a therapeutic value. Loeb²¹ and

¹² Science, 1905, 21, p. 611.

¹³ Jour. Infect. Dis., 1906, Suppl. 2, p. 175.

¹⁴ Deutsch. med. Wchnschr., 1913, 39, p. 549.

¹⁵ Münch. med. Wchnschr., 1914, 61, p. 586.

¹⁶ Jour. Infect. Dis., 1914, 15, p. 245.

¹⁷ Quoted without reference by Pfeffer, Physiology of Plants, Eng. ed., 1903, 2, pp. 260, 262, 266.

¹⁸ Handbuch d. chemische Mittel gegen Pflanzenkrankheiten, 1898, p. 75.

¹⁹ Ztschr. f. Medizinalbeamte., 1913, 26, p. 772.

²⁰ Jour. Ind. and Eng. Chem., 1909, 1, p. 676.

²¹ Jour. Am. Med. Assn., 1913, 60, p. 1857. Jour. Exper. Med., 1914, 20, pp. 169, 180, 503, 522. Arch. f. Int. Med., 1915, 15, p. 974.

his co-workers are experimenting with injections of colloidal copper in the treatment of cancer, while von Linden and her co-workers have reported favorably on the action of copper in the treatment of tuberculosis. Luton²² insists on the therapeutic value of copper compounds in the form of pills, salves, or injections, in all kinds of tuberculous infections, however severe. According to his statement the use of copper for the treatment of tuberculosis dates back to 1885, and has continued spasmodically ever since, frequently effecting a cure in the early stages and exercising a palliative effect under all circumstances. Corper,²³ however, found that copper salts injected into tuberculous guinea-pigs or rabbits had no effect on the course of the disease, a statement confirmed by Moewes and Jauer,²⁴ by Kaiser,²⁵ and others.

A large part of the prejudice against the use of copper, especially for the purification of drinking water, is due, apart from any unpleasant flavor which may result, to a general notion that copper is poisonous to animals. Investigation has proved that this metal is constantly present in the animal body, and occurs naturally in a large number of those foods which combine to form the usual diet of mankind. That its presence in the diet is practically harmless has been held by many physiologists. Galippe²⁶ and Kraemer,¹ by using copper utensils for the preparation and serving of food, for periods of many months, demonstrated the innocuousness of small amounts of copper in the diet. Many pharmacologists regard it as innocuous, and Holland, in correspondence with Kraemer, stated that to produce poisoning the administration of copper must be deliberate and in large amounts, and that the physical action would cease with cessation of the application of the salt.

The present work, which was undertaken because of the conflicting testimony of investigators as to the bactericidal power of copper, falls naturally into two divisions: first, investigation of the killing power of the sulfate and the chlorid of copper, and second, a study of their inhibitory action. The organisms used have been chiefly bacteria—*B. coli*, *B. typhosus*, *B. prodigiosus*, *B. tuberculosis*, and *Staphylococcus aureus*; but for comparison the behavior of certain fungi (a baker's white yeast, a pink torula, *Aspergillus niger*, and *Penicillium decumbens*), was also observed.

TECHNIC

In the earlier experiments Krönig and Paul's garnet method was followed: A suspension of organisms was allowed to dry on glass beads, which were then exposed to the action of various dilutions of copper. After washing with ammonium sulfite to neutralize any adherent copper solution, and then with water, a definite number of garnets were placed in test tubes, thoroughly shaken in a shaking machine, and then plated with 10 c.c. of agar. More consistent results were obtained with a modification of the method devised by Anderson and McClintock, which was accordingly substituted for the garnet method in the later experiments. The procedure was as follows: The growth

²² La Province med., 1912, 23, p. 549.

²³ Jour. Infect. Dis., 1914, 15, p. 518.

²⁴ München. med. Wchnschr., 1914, 61, p. 1439.

²⁵ Therap. Monatsh., 1914, 28, p. 748.

²⁶ Ann. d'hyg. pub., 1878, 50, p. 426.

from a 48-hour agar-slant culture of the organism was mixed with sterile normal salt solution, or with sterile water distilled in glass, to give a faintly opaque suspension. Of this suspension 0.1 c.c. was added to 15 c.c. of each of the copper dilutions and left for various lengths of time, exposures of 2½, and 15 minutes being finally selected for the experimental routine, tho in certain experiments the time was extended to 2, 4, and even 24 hours. One drop of the inoculated copper solution was then placed in a tube containing 10 c.c. of sterile distilled water and 1 c.c. of this dilution was used with 10 c.c.

TABLE 1

AVERAGE RESULTS OF 15-MINUTE EXPOSURES OF VARIOUS ORGANISMS TO THE ACTION OF COPPER

| Organism | CuCl ₂ | | | | | |
|------------------------------------|-------------------|-------|-------|-------|--------|---------|
| | 5% | 1% | 0.1% | 0.01% | 0.001% | 0.0001% |
| <i>Staphylococcus aureus</i> | 0 | 705 | 4,752 | 5,792 | 11,824 | 16,899 |
| <i>Bacillus coli</i> | 2 | 3 | 16 | 868 | 294 | 16,962 |
| <i>Bacillus prodigiosus</i> | 0 | 0 | 1 | 0 | 1 | 1,930 |
| Yeast (white)..... | 0 | 63 | 67 | 318 | 2,214 | 5,874 |
| <i>Torula</i> (pink)..... | 0 | 94 | 129 | 703 | 1,036 | 7,168 |
| <i>Aspergillus</i> | 278 | 798 | 948 | 1,043 | 1,132 | |
| <i>Penicillium decumbens</i> | 615 | 1,211 | | 1,771 | | 2,112 |

TABLE 2

THE EXTENT OF VARIATION IN THE ACTION OF COPPER ON *STAPHYLOCOCCUS AUREUS* AND ON *BACILLUS COLI* IN AN EXPOSURE OF 15 MINUTES' DURATION

| Experiment | | CuCl ₂ | | | | | |
|------------------------|--------|-------------------|-------|--------|--------|--------|---------|
| | | 5% | 1% | 0.1% | 0.01% | 0.001% | 0.0001% |
| <i>S. aureus</i> | 1..... | | | | | | |
| | 2..... | | | | | | |
| | 3..... | | | | | | |
| | 4..... | | | | | | |
| | 5..... | | | | | | |
| | 6..... | | | | | | |
| | 7..... | 0 | 0 | 0 | 36 | 6,391 | 1,496 |
| | 8..... | 0 | 2,116 | 14,256 | 17,339 | 23,142 | 38,184 |
| | 9..... | 0 | 0 | 0 | 1 | 5,940 | 11,016 |
| <i>B. coli</i> | 1..... | | | | | | |
| | 2..... | | | | | | |
| | 3..... | | | | | | |
| | 4..... | | | | | | |
| | 5..... | 0 | 0 | 14 | 21 | 911 | 42,370 |
| | 6..... | 1 | 1 | 28 | 3,210 | 77 | 24,124 |
| | 7..... | 0 | 1 | 12 | 239 | 178 | 921 |
| | 8..... | 6 | 10 | 9 | 3 | 10 | 433 |

of agar, for each of 4 plates. The further dilution of the inoculated copper solution by transfer to 10 c.c. of water instead of directly to the plate, was designed to avoid any inhibitory effect which might result from the presence of so large an amount of copper in the agar, since in these experiments it was the killing action of copper which was of primary interest. Controls were treated in the same way throughout, sterile distilled water or sterile normal salt solution being substituted for the copper dilution.

For convenience in the comparison of results, the amounts of either salt used in making the dilutions were so calculated as to give corresponding per-

centages of copper. Dilutions containing 5, 1, 0.1, 0.01, 0.001, 0.0001% of copper were used.

All plates were counted after 48 hours in the incubator or at room temperature, according to the organism.

In Table 1 are given the average results of the various experiments with the different organisms (by the Anderson and McClintock method, modified). It will be observed that the organisms manifested a certain

TABLE 1—*Continued*
AVERAGE RESULTS OF 15-MINUTE EXPOSURES OF VARIOUS ORGANISMS TO THE ACTION OF COPPER

| CuSO ₄ | | | | | | | Control 0.9% NaCl |
|-------------------|-------|-------|-------|--------|---------|----------|----------------------|
| 5% | 1% | 0.1% | 0.01% | 0.001% | 0.0001% | 0.00001% | |
| 86 | 389 | 4,838 | 2,415 | 9,730 | 17,554 | 4,326 | 12,208 |
| 2 | 70 | 211 | 1,541 | 3,826 | 26,379 | 33,208 | 20,589 |
| 0 | 0 | 1 | 2 | 38 | 24,116 | 84,213 | 45,072 |
| 0 | 5 | 132 | 135 | 777 | 4,341 | 6,116 | 4,589 |
| 0 | 645 | 831 | 1,537 | 1,483 | 2,782 | 4,061 | 5,988 |
| 405 | 495 | 286 | 386 | 1,134 | 1,071 | | 689 |
| 646 | 1,096 | | 1,284 | | 1,855 | | 1,949 |

TABLE 2—*Continued*
THE EXTENT OF VARIATION IN THE ACTION OF COPPER ON STAPHYLOCOCCUS AUREUS AND ON
BACILLUS COLI IN AN EXPOSURE OF 15 MINUTES' DURATION

| CuSO ₄ | | | | | | | Control |
|-------------------|-------|--------|--------|--------|---------|----------|---------|
| 5% | 1% | 0.1% | 0.01% | 0.001% | 0.0001% | 0.00001% | |
| 103 | 240 | 228 | 337 | 490 | 6,967 | 580 | 874 |
| 214 | 168 | 16,518 | | | | | 10,928 |
| 29 | 63 | 57 | | | | | 578 |
| 0 | 0 | 2,549 | 6,312 | 18,969 | 28,141 | 8,072 | 10,076 |
| 2 | 960 | 5,143 | 11,584 | 14,236 | 22,448 | 12,936 | 5,790 |
| 2,137 | 3,175 | 22,176 | 42,078 | 31,860 | 57,548 | 37,175 | 8,946 |
| | | | | | | | 12,000 |
| | | | | | | | 46,528 |
| | | | | | | | 18,834 |
| 0 | 0 | 3 | 26 | 204 | 479 | 744 | 1,091 |
| 0 | 1 | 5 | 90 | 1,284 | 37,591 | 39,312 | 39,924 |
| 0 | 45 | 1 | 5,994 | 11,414 | 55,004 | 58,000 | 39,857 |
| 6 | 235 | 33 | 56 | 2,403 | 12,345 | 42,776 | 28,392 |
| | | | | | | | 30,919 |
| | | | | | | | 23,014 |
| | | | | | | | 1,030 |
| | | | | | | | 490 |

specificity in their reaction to copper, some being more susceptible than others. *B. prodigiosus*, for instance, appears little resistant to the action of copper, bacilli being practically all killed by dilutions of copper, as the sulfate, up to 1:10,000, and as the chlorid, up to 1:100,000; while *Staphylococcus aureus* is relatively resistant, 1% copper as chlorid and 5% as sulfate failing to kill every organism. With the exception of *Staphylococcus*, *Aspergillus*, and *Penicillium*,

5% copper in either form killed all the organisms studied. A slight difference in efficiency in favor of the chlorid is noticeable between the two salts.

That *Staphylococcus aureus* is a relatively resistant organism was noted by Green, who found that it succumbed to 2.5% copper chlorid (0.9% copper) only after 5 hours' exposure and to the same percentage of copper sulfate (0.6% copper) after 1 day. Bolton observed the same resistance; for 50 minutes' contact of metallic copper and an inoculated agar plate was required in the case of *Staphylococcus aureus* to produce a clear zone, while from 1 to 5 minutes' exposure was sufficient in the case of *B. coli*, *B. typhosus*, and *V. cholerae*. Furthermore, according to Bolton, the resistance of the same organism varied at

TABLE 3
RESULTS OF THE INHIBITORY ACTION OF COPPER ON VARIOUS MICRO-ORGANISMS

| Organism | CuCl ₂ | | | |
|-------------------------------------|-------------------|--------|--------|---------|
| | 0.1% | 0.01% | 0.001% | 0.0001% |
| <i>Staphylococcus aureus</i> | 0 | 0 | 3,584 | 8,239 |
| <i>Staphylococcus citreus</i> | 0 | 0 | 21,616 | 24,916 |
| <i>Bacillus coli</i> | 0 | 16,613 | 47,839 | 39,931 |
| <i>Bacillus prodigiosus</i> | 0 | 12,634 | 15,023 | 15,915 |
| <i>Yeast (white)</i> | 0 | 9,731 | 11,267 | 11,725 |
| <i>Torula (pink)</i> | 0 | 11,299 | 25,900 | 18,420 |
| <i>Aspergillus</i> | 0 | 204 | 167 | 157 |
| <i>Penicillium</i> | 0 | 1,142 | 1,762 | 1,502 |
| <i>B. tuberculosis</i> | 0 | 0 | 0 | +++ |

different times—a phenomenon met with in the experiments here reported. Table 2 illustrates the extent of variation in 9 experiments with *Staphylococcus aureus* and 8 with *B. coli*. While there is a general similarity of behavior from experiment to experiment, there are irregularities of progression and variations in the results obtained under apparently identical conditions (even 5% copper failing to kill *Staphylococcus aureus* in one instance)—variations which are largely obliterated on averaging the results.

For the study of the inhibitory action of copper such amounts of stock solutions of the chlorid and the sulfate were taken as, added to the 10 c.c. of agar used for plating, would give the desired dilution. (These amounts were introduced into the plate rather than added to the tube of agar, since precipitation of copper occurred on contact of the copper solution and the agar and only by rapid cooling after mixing could this precipitation be prevented or reduced to a minimum.) One-tenth cubic centimeter of the suspension of the organism was then

added. To avoid any preliminary inhibitory action of copper on the organism before the introduction of the agar not only were the copper dilution and the bacterial suspension introduced as far apart as the limit of the plate would permit, but the dilution was so calculated as to make the required amounts of stock solution as minute as was consistent with accuracy of measurement (0.1 or 0.2 c.c.). In the experiments for inhibitory action the dilutions contained 0.1, 0.01, 0.001, and 0.0001% copper.

Table 3 contains the average results of these experiments. It is obvious that, in general, inhibition occurs with a lower concentration of copper than that at which killing occurs. *B. tuberculosis*, which was included in this table, is more susceptible than the other organisms

TABLE 3—*Continued*
RESULTS OF THE INHIBITORY ACTION OF COPPER ON VARIOUS MICRO-ORGANISMS

| CuSO ₄ | | | | Control |
|-------------------|-------|--------|---------|---------|
| 0.1% | 0.01% | 0.001% | 0.0001% | |
| 0 | 0 | 0 | 47,178 | 17,870 |
| 0 | 0 | 39,288 | 29,792 | 33,509 |
| 0 | 0 | 45,305 | 59,961 | 46,252 |
| 0 | 0 | 71,718 | 69,708 | 34,914 |
| 0 | 0 | 13,800 | 12,372 | 19,072 |
| 0 | 1,628 | 30,816 | 20,484 | 18,948 |
| 0 | 150 | 212 | 95 | 180 |
| 0 | 1,707 | 1,758 | 2,222 | 1,264 |
| 0 | 0 | 0 | +++ | +++ |

used, its growth being inhibited by dilutions of 1 part copper, in either form, to 100,000 of media. Next in order, *Staphylococcus aureus* is inhibited by copper as chlorid in dilution of 1:10,000 and by copper as sulfate in dilution of 1:100,000. With the exception of *Penicillium*, against which the chlorid is less efficient than the sulfate, and of *Aspergillus*, on which the two salts have an equal effect, copper in the form of the sulfate has a stronger inhibitory action than in the form of the chlorid. Dilutions of 1:1000 inhibit growth of all the organisms. Dilutions of 1:10,000 inhibit *Staphylococcus aureus*, *B. coli*, *B. prodigiosus*, and the white yeast. In the case of the pink yeast and the two moulds the growth is diminished somewhat, but inhibited only by the stronger concentration.

It is noticeable that there is an exception to the rule that inhibition is accomplished by a lower concentration than is required to kill. *B. prodigiosus* is inhibited by dilutions of copper of 1:1000 as chlorid, and of 1:10,000 as sulfate, while dilutions of copper of 1:100,000 as

chlorid, and of 1:10,000 as sulfate, kill in 15 minutes. Since, however, repeated experiments give the same result, the peculiarity appears to be intrinsic in the organism. *B. coli* also forms a partial exception to the rule.

Inasmuch as the results thus far given were less favorable as regards the disinfectant value of copper than were the results in Moore's work,¹¹ other experiments were undertaken to see whether even under laboratory conditions, with longer exposures, comparable results might be obtained. Accordingly, 200 c.c. of ordinary tap water which had been allowed to flow for one-half hour were placed in each of several sterile

TABLE 4
RESULTS OF THE ACTION OF COPPER ON THE BACTERIAL CONTENT OF TAP WATER

| Time of Exposure | | CuCl ₂ | | | | |
|------------------------------|-------------|-------------------|-------|---------|---------|----------|
| | | 0.1% | 0.01% | 0.001% | 0.0001% | 0.00001% |
| In sterile flasks... | 1 hr. | 0 | 3 | 4 | 45 | 1,710 |
| | 3 hr. | 0 | 3 | 9 | 3,759 | 31,287 |
| | 24 hr. | 0 | 12 | 4 | 333 | 7,232 |
| | 48 hr. | 0 | 1 | 4 | 3,924 | 7,429 |
| | 96 hr. | 0 | 1 | 14 | 25,459 | 92,926 |
| | 1 wk. | 0 | 2 | 21,325 | 100,753 | 69,691 |
| | 2 wk. | 0 | 10 | 1,267 | 7,990 | 125,390 |
| | 3 wk. | 0 | 2 | 7,082 | 2,873 | 37,569 |
| | 4 wk. | 0 | 10 | 1,243 | 33,828 | 127,311 |
| | 5 wk. | 2 | 4 | 1,009 | 25,208 | 243,915 |
| | 6 wk. | 0 | 3 | 37,041 | 29,989 | 857,600 |
| In large open crocks.. | 1 hr. | 0 | 14 | 371 | 13,819 | 14,553 |
| | 3 hr. | 1 | 32 | 714 | 5,306 | 11,760 |
| | 24 hr. | 1 | 88 | 237 | 4,143 | 19,729 |
| | 48 hr. | 1 | 61 | 106 | 10,035 | 15,797 |
| | 96 hr. | 0 | 8 | 3,894 | 19,699 | 31,060 |
| | 1 wk. | 3 | 15 | 167,039 | 25,049 | 104,803 |
| | 2 wk. | 0 | 151 | 169,958 | 62,847 | 227,563 |

flasks, and sufficient amounts of stock solutions of copper chlorid and of copper sulfate added to make dilutions containing 0.1, 0.01, 0.001, 0.0001, and 0.00001% copper. The number of organisms present was obtained by making a control of the water alone. Plates were made after 1, 3, 24, and 96 hours, and after 1, 2, 3, 4, 5, and 6 weeks. Similarly, to approximate even more closely the conditions in an open reservoir, large earthen crocks, holding approximately 4 liters, were filled with tap water and the requisite amounts of chlorid or of sulfate added to make the dilutions desired. The crocks were left uncovered for 2 weeks in a sunny laboratory. The results are embodied in Table 4.

While 1 part copper to 1000 of water kills all the ordinary water organisms, and 1 part copper to 10,000 of water causes a great reduction in their number, they are little affected by higher dilutions of cop-

per, increasing in number rather than decreasing on long exposure. The ordinary water organisms, however, are relatively innocuous; it is only the pathogenic bacteria the presence of which in water is of special sanitary significance. Is copper as toxic to these as Moore¹¹ and his contemporary workers claimed? To test this point 0.5 c.c. of a suspension of *B. coli* was added to flasks containing sterile tap water and the same amounts of copper as before, and plates were made at intervals, up to 3 weeks. From Table 5 it is seen that copper as sulfate in dilutions of 1:1,000,000 killed all organisms in 24 hours, and in dilutions of 1:10,000,000, as both sulfate and chlorid, in 96 hours.

TABLE 4—*Continued*
RESULTS OF THE ACTION OF COPPER ON THE BACTERIAL CONTENT OF TAP WATER

| CuSO ₄ | | | | | Control |
|-------------------|-------|---------|---------|----------|---------|
| 0.1% | 0.01% | 0.001% | 0.0001% | 0.00001% | |
| 0 | 2 | 4 | 41 | 6,505 | 8,835 |
| 0 | 1 | 4 | 3,533 | 41,104 | 17,037 |
| 0 | 8 | 15 | 173 | 9,223 | 78,983 |
| 0 | 2 | 2 | 2,079 | 12,708 | 23,690 |
| 0 | 1 | 198 | 40,427 | 68,051 | 15,138 |
| 0 | 3 | 34,504 | 62,782 | 39,278 | 21,103 |
| 0 | 15 | 6,554 | 192,288 | 75,993 | 38,458 |
| 0 | 1 | 19,863 | 106,062 | 67,128 | 15,299 |
| 0 | 7 | 21,293 | 181,261 | 91,651 | 29,961 |
| 2 | 35 | 24,116 | 124,933 | 137,697 | 56,522 |
| 0 | 1 | 1,642 | 124,336 | 617,876 | 114,746 |
| 0 | 11 | 1,325 | 6,517 | 28,195 | 14,374 |
| 1 | 13 | 63 | 3,477 | 9,221 | 16,732 |
| 1 | 15 | 13 | 8,174 | 20,025 | 7,984 |
| 1 | 11 | 38 | 7,915 | 16,140 | 3,589 |
| 0 | 8 | 306 | 14,607 | 18,290 | 8,518 |
| 2 | 31 | 23,301 | 36,864 | 105,795 | 5,197 |
| 0 | 47 | 129,601 | 55,460 | 279,288 | 6,888 |

In making the experiments it was observed that a heavy precipitation occurred on the addition of the copper to the tap water, making it impossible to tell the actual amount of copper in a solution producing a given effect. To overcome this difficulty, distilled water was used instead of tap water, but since controls in distilled water died, the presence of traces of copper was suspected. Analyses of the water verified this suspicion. That ordinary distilled water contained traces of metals, particularly of copper, had been noticed by Ringer, who ascribed the toxic action of distilled water on living organisms to this constituent. For this reason, in subsequent experiments water redistilled in glass was employed. In twice-distilled water the controls lived indefinitely, plates made 8 weeks after inoculation showing but slight reduction. Colon bacilli exposed to various amounts of copper failed to survive for 24 hours in dilutions of 1:1,000,000.

The length of time required for complete sterilization of twice-distilled water in dilutions of 1:1,000,000, as compared with that required for tap water in the same dilution, suggests the possible presence of copper ordinarily in the latter. Analyses made in the chemical laboratory of the University of Chicago showed this to be a fact.

TABLE 5
RESULTS OF THE ACTION OF COPPER ON B. COLI IN STERILE TAP WATER, AND IN TWICE-DISTILLED WATER

| Time of Exposure | | CuCl ₂ | | | | |
|--------------------------|--------------|-------------------|--------|---------|---------|----------|
| | | 0.1% | 0.01% | 0.001% | 0.0001% | 0.00001% |
| In sterile tap water | 1 hr. | 0 | 1,403 | 229,517 | 613,651 | 781,151 |
| | 3 hr. | 0 | 4 | 88,602 | 96,765 | 566,282 |
| | 24 hr. | 0 | 0 | 316 | 440 | 69,616 |
| | 48 hr. | 0 | 0 | 0 | 0 | 5,682 |
| | 96 hr. | 0 | 0 | 0 | 0 | 0 |
| | 1 wk. | 0 | 0 | 0 | 0 | 0 |
| | 2 wk. | 0 | 0 | 0 | 0 | 0 |
| | 3 wk. | 0 | 0 | 0 | 0 | 0 |
| In twice distilled water | 30 min. | 236 | 42,437 | 60,648 | 315,309 | 981,441 |
| | 1 hr. | 9 | 2,881 | 36,707 | 668,427 | 695,331 |
| | 3 hr. | 0 | 69 | 23 | 9,567 | 757,266 |
| | 24 hr. | 0 | 0 | 0 | 0 | 22,536 |
| | 48 hr. | 0 | 0 | 0 | 0 | 4,472 |
| | 1 wk. | 0 | 0 | 0 | 0 | 87 |
| | 2 wk. | 0 | 0 | 0 | 0 | 1 |
| | 3 wk. | 0 | 0 | 0 | 0 | 0 |
| | 4 wk. | | | | | |
| | 5 wk. | | | | | |
| | 6 wk. | | | | | |
| | 7 wk. | | | | | |
| | 8 wk. | | | | | |

TABLE 6
RESULTS OF THE ACTION OF COPPER ON NATURAL B. COLI IN TWICE-DISTILLED WATER (GLASS), AND IN STERILE TAP WATER

| Time of Exposure | | CuCl ₂ | | | | |
|--------------------------|-------------|-------------------|-------|---------|---------|-----------|
| | | 0.1% | 0.01% | 0.001% | 0.0001% | 0.00001% |
| In twice distilled water | 1 hr. | 0 | 0 | 2 | 94,793 | 1,224,496 |
| | 3 hr. | 0 | 0 | 0 | 32,714 | 579,080 |
| | 24 hr. | 0 | 0 | 0 | 1 | 355,327 |
| | 48 hr. | 0 | 0 | 0 | 0 | 291,986 |
| | 96 hr. | 0 | 0 | 0 | 0 | 242,402 |
| | 1 wk. | 0 | 0 | 0 | 0 | 200,896 |
| | 2 wk. | 0 | 0 | 0 | 0 | 186,192 |
| In sterile tap water | 1 hr. | 0 | 35 | 151,024 | 569,401 | 831,046 |
| | 3 hr. | 0 | 2 | 77,763 | 100,606 | 665,801 |
| | 24 hr. | 0 | 1 | 8 | 36 | 95,111 |
| | 48 hr. | 0 | 0.6 | 0 | 26 | 184,563 |
| | 96 hr. | 0 | 0 | 0 | 26 | 198,146 |
| | 1 wk. | 0 | 0 | 0 | 4 | 55,946 |
| | 2 wk. | 0 | 0 | 0 | 0 | 8,914 |

Since Clark and Gage¹³ had found that B. coli living in water resisted the action of copper sulfate in dilution of 1:10,000 for 103 days, while laboratory cultures were killed in 24 hours, our experiment

was repeated with a strain of *B. coli* recently isolated from drinking water. Their statement appears justifiable, since (Table 6) this water strain of *Bacillus coli* survived for 1 week in dilutions of 1:1,000,000, and when the experiment was brought to an end, after 2 weeks, the organisms were still numerous, tho their number was much reduced,

TABLE 5—*Continued*
RESULTS OF THE ACTION OF COPPER ON *B. COLI* IN STERILE TAP WATER, AND IN TWICE-DISTILLED WATER

| CuSO ₄ | | | | | Control |
|-------------------|-------|---------|---------|----------|-----------|
| 0.1% | 0.01% | 0.001% | 0.0001% | 0.00001% | |
| 0 | 5,534 | 259,765 | 560,258 | 740,182 | 1,155,975 |
| 0 | 2 | 22,133 | 17,121 | 297,777 | 1,118,864 |
| 0 | 0 | 0 | 0 | 34,720 | 1,191,685 |
| 0 | 0 | 0 | 0 | 5,299 | 672,956 |
| 0 | 0 | 0 | 0 | 0 | 1,783,621 |
| 0 | 0 | 0 | 0 | 0 | 588,351 |
| 0 | 0 | 0 | 0 | 0 | 530,643 |
| 0 | 0 | 0 | 0 | 0 | 443,808 |
| 30,101 | 6,841 | 15,992 | 88,104 | 603,104 | 1,389,925 |
| 219 | 1,011 | 1,760 | 32,766 | 843,831 | 1,395,305 |
| 0 | 9 | 21 | 2,158 | 324,191 | 1,204,917 |
| 0 | 0 | 0 | 0 | 7,480 | 1,263,993 |
| 0 | 0 | 0 | 0 | 1,024 | 1,159,845 |
| 0 | 0 | 0 | 0 | 16 | 1,027,096 |
| 0 | 0 | 0 | 0 | 0 | 857,784 |
| 0 | 0 | 0 | 0 | 0 | 968,931 |
| | | | | | 1,157,815 |
| | | | | | 1,222,720 |
| | | | | | 1,310,481 |
| | | | | | 739,159 |
| | | | | | 208,765 |

TABLE 6—*Continued*
RESULTS OF THE ACTION OF COPPER ON NATURAL *B. COLI* IN TWICE-DISTILLED WATER (GLASS), AND IN STERILE TAP WATER

| CuSO ₄ | | | | | Control |
|-------------------|-------|---------|---------|-----------|-----------|
| 0.1% | 0.01% | 0.001% | 0.0001% | 0.00001% | |
| 0 | 0 | 1 | 104,286 | 1,455,248 | 3,971,680 |
| 0 | 0 | 1 | 39,588 | 784,973 | 3,785,953 |
| 0 | 0 | 2 | 2 | 381,606 | 4,194,546 |
| 0 | 0 | 0 | 0 | 401,923 | 4,680,873 |
| 0 | 0 | 0 | 0 | 464,165 | 4,192,560 |
| 0 | 0 | 0 | 0 | 374,110 | 1,655,240 |
| 0 | 0 | 0 | 0 | 99,788 | 1,478,666 |
| 0 | 40 | 233,008 | 530,951 | 867,479 | 1,187,658 |
| 0 | 12 | 9,652 | 105,388 | 680,907 | 1,389,104 |
| 0 | 0 | 1 | 11 | 82,106 | 1,411,281 |
| 0 | 0 | 2 | 3 | 184,343 | 1,435,859 |
| 0 | 0 | 0.6 | 6 | 110,294 | 1,418,373 |
| 0 | 0 | 0.5 | 1 | 37,923 | 1,473,970 |
| 0 | 0 | 0 | 0 | 25,054 | 1,575,318 |

in a dilution of 1:10,000,000. In twice-distilled water, 24 hours' exposure to copper in dilutions up to 1:1,000,000 was fatal. In 1:10,000,000 dilution the organisms were still living, tho reduced in

number, after 2 weeks. Our water strain of *B. coli*, therefore, shows no such marked resistance as was reported by Clark and Gage.³

Moore¹¹ made the statement that *B. typhosus* was even more easily killed than *B. coli*, on exposure to copper. Table 7 gives the results of experiments with *B. typhosus*. In tap water this organism, like *B. coli*, continued alive in dilutions of 1:10,000,000 after 2 weeks' exposure. In a higher concentration, 96 hours' exposure killed. In twice-distilled water, all dilutions, even 1:10,000,000, killed in 24 hours. The difference in susceptibility between our strains of these organisms is therefore very slight.

TABLE 7
RESULTS OF THE ACTION OF COPPER ON *B. TYPHOSUS* IN TWICE-DISTILLED WATER (GLASS), AND
IN STERILE TAP WATER

| Time of Exposure | | CuCl ₂ | | | | |
|--------------------------|-------------|-------------------|-------|--------|---------|-----------|
| | | 0.1% | 0.01% | 0.001% | 0.0001% | 0.00001% |
| In twice distilled water | 1 hr. | 0 | 1 | 2 | 115 | 226,920 |
| | 3 hr. | 0 | 0 | 0.5 | 3 | 798 |
| | 24 hr. | 0 | 0 | 0 | 0 | 0.5 |
| | 48 hr. | 0 | 0 | 1 | 1 | 0 |
| | 96 hr. | 0 | 0 | 0 | 0 | 0 |
| | 1 wk. | 0 | 0 | 0 | 0 | 0 |
| | 2 wk. | 0 | 0 | 0 | 0 | 0 |
| In sterile tap water | 1 hr. | 0 | 2 | 2,509 | 44,445 | 1,170,870 |
| | 3 hr. | 0 | 18 | 76 | 272 | 249,118 |
| | 24 hr. | 0 | 0 | 6 | 3 | 5 |
| | 48 hr. | 0 | 0 | 3 | 4 | 45 |
| | 96 hr. | 0 | 0 | 0 | 0 | 101 |
| | 1 wk. | 0 | 0 | 0 | 0 | 36 |
| | 2 wk. | 0 | 0 | 0 | 0 | 43 |

CONCLUSIONS

In the short time of an ordinary laboratory experiment and with the small amount of material usually employed, copper is unreliable and unsatisfactory both as a bactericide and as a fungicide. One percent frequently fails to kill all organisms within 15 minutes. As a rule, the degree of sterilization is proportional to the concentration of the copper and to the time of exposure. Only slight differences can be observed between the chlorid and the sulfate, this fact suggesting that such germicidal action as is present is dependent on the copper ion.

A certain specificity is apparent, some organisms (notably *B. prodigiosus*) being markedly susceptible to the effect of copper, while others (as *Staphylococcus aureus* and the moulds) are very resistant, surviving 15 minutes' exposure to even 5% solution.

As is to be expected, it usually requires lower concentrations for inhibition of growth than for killing, tho *B. prodigiosus* and to some extent *B. coli* seem to form an exception to this rule.

Our experiments indicate that in the purification of contaminated water supplies, with no limit as to time and with usually relatively few organisms distributed through a large amount of water, copper performs its function as a germicide much more efficiently than in the short-time experiment. Whether in closed sterile flasks or in large open vessels exposed to air and sunlight, it causes a diminution in the number of the ordinary water organisms and kills such bacteria as *B. coli* and *B. typhosus* (the most common pathogenic contaminators of water supplies) when in concentration of 1 part of the metal to 1,000,000 parts of the water—a concentration which could have no

TABLE 7—*Continued*
RESULTS OF THE ACTION OF COPPER ON *B. TYPHOSUS* IN TWICE-DISTILLED WATER (GLASS), AND
IN STERILE TAP WATER

| CuSO ₄ | | | | | Control |
|-------------------|-------|--------|---------|-----------|-----------|
| 0.1% | 0.01% | 0.001% | 0.0001% | 0.00001% | |
| 0 | 0.3 | 0.6 | 10,364 | 961,370 | 3,929,420 |
| 0 | 0.8 | 0.5 | 355 | 10,487 | 2,768,854 |
| 0 | 0 | 0.3 | 0 | 2 | 3,082,320 |
| 0 | 0 | 0 | 1 | 1 | 3,021,840 |
| 0 | 0 | 0 | 0 | 0 | 1,392,720 |
| 0 | 0 | 0 | 0 | 0 | 1,158,572 |
| 0 | 0 | 0 | 0 | 0 | 97,280 |
| 0 | 17 | 779 | 935 | 1,140,578 | 1,855,917 |
| 0 | 17 | 32 | 69 | 1,049,368 | 1,647,189 |
| 0 | 2 | 0.05 | 24 | 344,146 | 1,742,329 |
| 0 | 2 | 29 | 23 | 167,253 | 1,919,152 |
| 0 | 0 | 0.6 | 0 | 163,118 | 1,413,774 |
| 0 | 0 | 0 | 0 | 117,813 | 2,437,054 |
| 0 | 0 | 0 | 0 | 3,336 | 934,776 |

injurious effect on those using the water. To obtain this concentration about 2.5 parts of copper chlorid to 1,000,000 of water and about 4 parts of copper sulfate to 1,000,000 of water must be used. The amount used, however, must be varied in accordance with the amount of organic matter present and with the degree of contamination of the water. For this reason copper salts are far less efficient in the purification of sewage.

The fact demonstrated that very low concentrations of copper will, if sufficient time is allowed, kill or inhibit the growth of many pathogenic organisms, would seem to suggest that copper salts, since their toxicity is low, should have a certain therapeutic value, especially in diseases caused by those bacteria which are easily killed or inhibited by the metal. Its prophylactic and therapeutic use in typhoid fever and cholera has been noted in the literature cited. It has but slight bactericidal action on the tubercle bacillus, but in the treatment of an infec-

tious disease an inhibitory action may be, and probably is, quite as important as complete destruction. As already noted, a dilution of 1:100,000 prevents the growth of tubercle bacilli in the test tube. If, then, this concentration could be attained and maintained in the animal body, we might be able to prevent the further development of the disease and eventually to kill the organisms. However, the experiments of Corper²⁸ with various copper salts, and of DeWitt²⁶ with copper trypan blue, seem to show that soluble copper salts, when introduced into the animal body, are changed into insoluble form and held for the most part at the point of injection, where they give rise to severe ulcerations and necrosis. If these salts are introduced by mouth, they are relatively innocuous; but Corper²⁸ found that, whether fed or injected, the salts used by him and also colloidal copper had no effect on the course of the tuberculous process; he was also unable to find any appreciable amounts of copper in the tuberculous glands or eyes. In other words, he was unable to show any specific affinity of copper for the tuberculous tissues.